

=> d his

(FILE 'HOME' ENTERED AT 09:10:29 ON 20 JUN 2003)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 09:10:42 ON 20 JUN 2003

SEA CELLULASE

-----  
2 FILE ADISCTI  
1 FILE ADISNEWS  
3385 FILE AGRICOLA  
135 FILE ANABSTR  
286 FILE AQUASCI  
1693 FILE BIOBUSINESS  
161 FILE BIOCOMMERCE  
8748 FILE BIOSIS  
6298 FILE BIOTECHABS  
6298 FILE BIOTECHDS  
2659 FILE BIOTECHNO  
5291 FILE CABA  
38 FILE CANCERLIT  
16462 FILE CAPLUS  
1907 FILE CEABA-VTB  
24 FILE CEN  
105 FILE CIN  
227 FILE CONFSCI  
166 FILE CROPB  
217 FILE CROPU  
76 FILE DDFB  
37 FILE DDFU  
3988 FILE DGENE  
76 FILE DRUGB  
75 FILE DRUGLAUNCH  
289 FILE DRUGMONOG2  
50 FILE DRUGU  
30 FILE EMBAL  
2956 FILE EMBASE  
1719 FILE ESBIODASE  
160 FILE FEDRIP  
64 FILE FOREGE  
825 FILE FROSTI  
2114 FILE FSTA  
1275 FILE GENBANK  
20 FILE HEALSAFE  
1290 FILE IFIPAT  
1713 FILE JICST-EPLUS  
2 FILE KOSMET  
3324 FILE LIFESCI  
3 FILE MEDICONF  
2807 FILE MEDLINE  
12 FILE NIOSHTIC  
351 FILE NTIS  
101 FILE OCEAN  
4968 FILE PASCAL  
10 FILE PHIN  
322 FILE PROMT  
20 FILE RDISCLOSURE  
6414 FILE SCISEARCH  
1 FILE SYNTHLINE  
1718 FILE TOXCENTER

BEST AVAILABLE COPY

4953 FILE USPATFULL  
143 FILE USPAT2  
10 FILE VETB  
215 FILE VETU  
2653 FILE WPIDS  
2653 FILE WPINDEX

L1

QUE CELLULASE  
-----

FILE 'CAPLUS, BIOSIS, SCISEARCH, BIOTECHDS, CABA, PASCAL, AGRICOLA,  
LIFESCI, EMBASE, MEDLINE, BIOTECHNO' ENTERED AT 09:11:54 ON 20 JUN 2003

L2 89 S L1 AND MARITIMA  
L3 60 S L2 AND (PURIF? OR ISOLAT? OR CLON?)  
L4 29 DUP REM L3 (31 DUPLICATES REMOVED)  
L5 1 S L4 AND (VARIANT OR MUTANT)

=> d l5 ibib ab

**BEST AVAILABLE COPY**

=> d 14 ibib ab 1-29

L4 ANSWER 1 OF 29 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 1  
ACCESSION NUMBER: 2002:964542 CAPLUS  
DOCUMENT NUMBER: 138:35298  
TITLE: **Cloning**, sequence and mutagenesis of  
Thermotoga **maritima** carboxymethyl  
**cellulase** gene, bioinformatics applications  
and use of the recombinant enzyme in food supplement  
or detergent, or for modulator screening  
INVENTOR(S): Short, Jay M.; Mathur, Eric J.; Lam, David E.  
PATENT ASSIGNEE(S): Diversa Corporation, USA  
SOURCE: PCT Int. Appl., 168 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 4  
PATENT INFORMATION:

**BEST AVAILABLE COPY**

| PATENT NO.   | KIND | DATE     | APPLICATION NO. | DATE     |
|--|------|----------|-----------------|----------|
| WO 2002101078  | A2   | 20021219 | WO 2002-US18782 | 20020612 |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,<br>CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,<br>GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,<br>LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,<br>PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,<br>UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU,<br>TJ, TM  |      |          |                 |          |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,<br>CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,<br>BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG   |      |          |                 |          |
| US 2003044956  | A1   | 20030306 | US 2001-880729  | 20010612 |
| PRIORITY APPLN. INFO.:<br>US 2001-880729 A2 20010612<br>US 1995-518615 A3 19950823<br>US 1997-951889 A3 19971016<br>US 1999-472857 A2 19991227   |      |          |                 |          |
| AB This invention provides hydrolases, e.g., <b>cellulase</b> enzymes,<br>polynucleotides encoding these enzymes, the use of such polynucleotides<br>and polypeptides and methods of making and using them. In one aspect, the<br>invention provides enzymes having carboxymethyl <b>cellulase</b><br>activity. <b>Cloning</b> of carboxymethyl <b>cellulase</b> gene<br>from Thermotoga <b>maritima</b> is described, and the genomic and<br>encoded amino acid sequences of the enzyme are disclosed. Mutagenesis of<br>the T. <b>maritima</b> carboxymethyl <b>cellulase</b> gene and<br>construction of transgenic non-human animals, plants and seeds is<br>described. Bioinformatics methods and construction of nucleic acid probes<br>and primers related to the T. <b>maritima</b> carboxymethyl<br><b>cellulase</b> gene are disclosed. The recombinant thermotolerant<br>carboxymethyl <b>cellulase</b> from T. <b>maritima</b> can be used<br>for screening of <b>cellulase</b> modulators, treating cellulose-contg.<br>fabric, pulp or paper products, in detergents, in food supplements or for<br>waste treatment. |      |          |                 |          |

L4 ANSWER 2 OF 29 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
ACCESSION NUMBER: 2002:210645 BIOSIS  
DOCUMENT NUMBER: PREV200200210645  
TITLE: Cellulolytic enzymes from Thermotoga species.  
AUTHOR(S): Liebl, Wolfgang (1)  
CORPORATE SOURCE: (1) Institut fuer Mikrobiologie und Genetik,  
Georg-August-Universitaet, D-37077, Goettingen Germany  
SOURCE: Adams, Michael W. W. [Editor]; Kelly, Robert M. [Editor].  
Methods in Enzymology, (2001) Vol. 330, pp. 290-300.

Methods in Enzymology. Hyperthermophilic enzymes: Part A.  
print.  
Publisher: Academic Press Inc. 525 B Street, Suite 1900,  
San Diego, CA, 92101-4495, USA.  
ISSN: 0076-6879. ISBN: 0-12-182231-1 (cloth).

DOCUMENT TYPE: Book  
LANGUAGE: English

L4 ANSWER 3 OF 29 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 2  
ACCESSION NUMBER: 2001379272 EMBASE  
TITLE: Galactomannanases Man2 and Man5 from Thermotoga species:  
Growth physiology on galactomannans, gene sequence  
analysis, and biochemical properties of recombinant  
enzymes.  
AUTHOR: Parker K.N.; Chhabra S.R.; Lam D.; Callen W.; Duffaud G.D.;  
Snead M.A.; Short J.M.; Mathur E.J.; Kelly R.M.  
CORPORATE SOURCE: R.M. Kelly, Department of Chemical Engineering, North  
Carolina State University, Box 7905, Raleigh, NC  
27695-7905, United States. rmkelly@eos.ncsu.edu  
SOURCE: Biotechnology and Bioengineering, (5 Nov 2001) 75/3  
(322-333).  
Refs: 73  
ISSN: 0006-3592 CODEN: BIBIAU  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 004 Microbiology  
029 Clinical Biochemistry  
LANGUAGE: English  
SUMMARY LANGUAGE: English

**BEST AVAILABLE COPY**

AB The enzymatic hydrolysis of mannan-based hemicelluloses is technologically important for applications ranging from pulp and paper processing to food processing to gas and oil well stimulation. In many cases, thermostability and activity at elevated temperatures can be advantageous. To this end, the genes encoding .beta.-mannosidase (man2) and .beta.-mannanase (man5) from the hyperthermophilic bacteria Thermotoga neapolitana 5068 and Thermotoga maritima were isolated, cloned, and expressed in Escherichia coli. The amino acid sequences for the mannosidases from these organisms were 77% identical and corresponded to proteins with an M(r) of approximately 92 kDa. The translated nucleotide sequences for the .beta.-mannanase genes (man5) encoded polypeptides with an M(r) of 76 kDa that exhibited 84% amino acid sequence identity. The recombinant versions of Man2 and Man5 had similar respective biochemical and biophysical properties, which were also comparable to those determined for the native versions of these enzymes in T. neapolitana. The optimal temperature and pH for the recombinant Man2 and Man5 from both organisms were approximately 90.degree.C and 7.0, respectively. The presence of Man2 and Man5 in these two Thermotoga species indicates that galactomannan is a potential growth substrate. This was supported by the fact that .beta.-mannanase and .beta.-mannosidase activities were significantly stimulated when T. neapolitana was grown on guar or carob galactomannan. Maximum cell densities increased by at least tenfold when either guar or carob galactomannan was added to the growth medium. For T. neapolitana grown on guar at 83.degree.C, Man5 was secreted into the culture media, whereas Man2 was intracellular. These localizations were consistent with the presence and lack of signal peptides for Man5 and Man2, respectively. The identification of the galactomannan degrading enzymes in these Thermotoga species adds to the list of biotechnologically important hemicellulases produced by members of this hyperthermophilic genera.  
.COPYRGT. 2001 John Wiley & Sons, Inc.

L4 ANSWER 4 OF 29 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 3  
ACCESSION NUMBER: 2001:125828 CAPLUS  
DOCUMENT NUMBER: 135:57649  
TITLE: Cellulolytic enzymes from Thermotoga species

AUTHOR(S): Liebl, Wolfgang  
CORPORATE SOURCE: USA  
SOURCE: Methods in Enzymology (2001), 330 (Hyperthermophilic Enzymes, Part A), 290-300  
CODEN: MENZAU; ISSN: 0076-6879  
PUBLISHER: Academic Press  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB .beta.-Glucans are important natural polymers consisting of .beta.-glycosidically linked glucose residues. These polysaccharides mostly fulfill structural roles. The .beta.-1,4-glucan cellulose and mixed-linkage .beta.-1,4/1,3-glucans, such as barley .beta.-glucan and lichenan, occur as cell wall components of higher plants and lichens, and .beta.-1,3-glucans are found in the cell walls of yeast and filamentous fungi and as structural and storage polysaccharide of the marine macroalga *Laminaria saccharina* (laminarin). Thermostable enzymes for the degrdn. of these types of .beta.-glucans and/or their corresponding genes have been **isolated** from different strains of the heterotrophic strictly anaerobic bacterial genus *Thermotoga*. The following is an overview of .beta.-glucan-cleaving enzymes found in *Thermotoga* species and describes the prepn. and characteristics of an endoglucanase and a .beta.-glucosidase of *Thermotoga maritima*. (c) 2001 Academic Press.

REFERENCE COUNT: 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 5 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI

ACCESSION NUMBER: 2001:514612 SCISEARCH

THE GENUINE ARTICLE: 444GE

TITLE: **Isolation** and cell wall regeneration of protoplasts from *Posidonia oceanica* and *Cymodocea nodosa*

AUTHOR: Balestri E (Reprint); Cinelli F

CORPORATE SOURCE: Dipartimento Sci Uomo & Ambiente, Via Volta 6, I-56100 Pisa, Italy (Reprint); Dipartimento Sci Uomo & Ambiente, I-56100 Pisa, Italy

COUNTRY OF AUTHOR: Italy

SOURCE: AQUATIC BOTANY, (JUL 2001) Vol. 70, No. 3, pp. 237-242.

Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE

AMSTERDAM, NETHERLANDS.

ISSN: 0304-3770.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 28

**BEST AVAILABLE COPY**

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB A method for the **isolation** of protoplasts from the seagrasses *Posidonia oceanica* and *Cymodocea nodosa* is described. **Isolation** of protoplasts was achieved using a combination of **cellulase** Onozuka R-10, hemicellulase and pectinase. **Purification** was carried out by Ficoll gradient centrifugation. A yield of  $1.87 \text{ (+/- } 0.16 \text{ SD)} \times 10^6$  protoplasts per gram of fresh tissue was obtained from mesophyll cells of *P. oceanica*. The viability of **isolated** protoplasts was 82.5% (+/- 10.6) as confirmed by fluorescein diacetate staining. A high percentage of protoplasts of *P. oceanica* (61.5 +/- 14.8%) regenerated the cell wall within 7 days as confirmed by staining with calcofluor white, but only a few protoplasts were able to divide. Callus-like structures were noticed after 20-30 days in culture. A lower yield of protoplasts,  $6.9 \text{ (+/- } 3.8) \times 10^5$  protoplasts per gram of fresh tissue, was obtained from mesophyll of *C. nodosa*. Viability of these protoplasts was 67.9% (+/- 12.6) after **isolation**. Some possible applications of the method are discussed. (C) 2001 Elsevier Science B.V. All rights reserved.

L4 ANSWER 6 OF 29 CAPLUS COPYRIGHT 2003 ACS

DUPLICATE 4

ACCESSION NUMBER: 2001:689911 CAPLUS

DOCUMENT NUMBER: 136:335862  
 TITLE: Identification and molecular characterization of an endoglucanase gene, *celS*, from the extremely thermophilic archaeon *Sulfolobus solfataricus*  
 AUTHOR(S): Limauro, Danila; Cannio, Raffaele; Fiorentino, Gabriella; Rossi, Mose; Bartolucci, Simonetta  
 CORPORATE SOURCE: Dipartimento di Chimica Organica e Biologica, Universita degli Studi di Napoli "Federico II", Naples, 80134, Italy  
 SOURCE: Extremophiles (2001), 5(4), 213-219  
 CODEN: EXTRFI; ISSN: 1431-0651  
 PUBLISHER: Springer-Verlag Tokyo  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 AB A genomic region upstream of the *alc.* dehydrogenase (*Ssadh*) gene was **cloned** and sequenced from a library of *Sulfolobus solfataricus* MT4 strain. The **isolated** 4,040-bp DNA fragment revealed an open reading frame (*celS*), lying in the opposite direction to *Ssadh*, which showed significant similarity to endo- $\beta$ -1,4-glucanases from *Pyrococcus furiosus*, *Thermotoga maritima*, and *T. neapolitana*. *celS* was shown to be a functional gene in vivo: a specific *celS* mRNA was detected by primer extension anal. showing a unique initiation transcription site coinciding with the ATG translation initiation codon. The specific gene product was detected as an extracellular **cellulase** after enzyme staining by CM-cellulose (CMC) SDS-PAGE, showing a mol. wt. in agreement with that deduced from the open reading frame. Depending on growth conditions, different levels of **cellulase** activity and specific *celS* transcript were detected, revealing an inductive effect of CMC and suggesting a repressive role of glucose.  
 REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 7 OF 29 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE  
 5

ACCESSION NUMBER: 2001:199142 BIOSIS  
 DOCUMENT NUMBER: PREV200100199142  
 TITLE: **Isolation** of leaf protoplasts from *Pancreatium maritimum* L. and two other dune plants: Possible applications.  
 AUTHOR(S): Balestri, Elena (1); Luccarini, Gualtiero; Cinelli, Francesco  
 CORPORATE SOURCE: (1) Centro Interuniversitario di Biologia Marina ed Ecologia Applicata, P.le Mascagni 1, 57126, Livorno Italy  
 SOURCE: Journal of Coastal Research, (Winter, 2001) Vol. 17, No. 1, pp. 188-194. print.  
 ISSN: 0749-0208.  
 DOCUMENT TYPE: Article  
 LANGUAGE: English  
 SUMMARY LANGUAGE: English

**BEST AVAILABLE COPY**

AB Protoplast techniques can be applied for studying physiological and biochemical processes in plant cells. In order to utilize these techniques, a method for **isolation** of viable protoplasts from dune plants was established. Using a combination of **cellulase** R-10 Onozuka, hemicellulase and pectinase protoplasts were **isolated** from younger leaves of *Pancreatium maritimum*. Field-grown plants harvested in autumn or winter yielded approximately six times as many protoplasts per g of fresh tissue as did plants collected in spring (3.6-5.3 X 10<sup>6</sup> versus 9 X 10<sup>5</sup> protoplasts per g of fresh tissue). No protoplasts were released from plants harvested in summer. The production of protoplasts from cultivated plants reached high yields of protoplasts (3.6 X 10<sup>6</sup>) independent to the season. The viability of these protoplasts was 89.2% (+1.1). Within 2-3 days of liquid culture 88.6% (+3.6) of the protoplasts were able to regenerate cell-walls. First divisions were

detected after 5-7 days of culture. By using the same procedure, yields of 2.31 X 10<sup>6</sup> and 1.28 X 10<sup>4</sup> protoplasts were obtained from field-collected leaves of *Cakile maritima* and *Ammophila arenaria*, respectively. Viability of protoplasts were 87.5% (+/-4.3) and 75.1% (+/-2.4), respectively. The potential applications of this method are discussed.

L4 ANSWER 8 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI  
ACCESSION NUMBER: 2000:527006 SCISEARCH  
THE GENUINE ARTICLE: 331RY  
TITLE: Mechanism of substrate hydrolysis by a thermophilic endoglucanase from *Thermotoga maritima*  
AUTHOR: Evans B R (Reprint); Gilman A K; Cordray K; Woodward J  
CORPORATE SOURCE: OAK RIDGE NATL LAB, DIV CHEM TECHNOL, OAK RIDGE, TN 37831 (Reprint)  
COUNTRY OF AUTHOR: USA  
SOURCE: BIOTECHNOLOGY LETTERS, (MAY 2000) Vol. 22, No. 9, pp. 735-740.  
Publisher: KLUWER ACADEMIC PUBL, SPUIBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS.  
ISSN: 0141-5492.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE; AGRI  
LANGUAGE: English  
REFERENCE COUNT: 23

**BEST AVAILABLE COPY**

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB A **cellulase** from the thermophile, *Thermotoga maritima*, hydrolyzed oligosaccharide substrates by an exoglucanase mode of action but acted as an endoglucanase to rapidly reduce the Viscosity of the soluble polysaccharides carboxymethylcellulose and barley beta-glucan. The V-max for hydrolysis of the substrate, p-nitrophenyl beta-D-cellobioside, was 42  $\mu$ mol min<sup>-1</sup> (mg protein)<sup>-1</sup>, while that for barley beta-glucan was 637. The enzyme had little activity on crystalline cellulose.

L4 ANSWER 9 OF 29 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
ACCESSION NUMBER: 2000:184666 BIOSIS  
DOCUMENT NUMBER: PREV200000184666  
TITLE: The mechanism of **cellulase** action on cotton fibers: Evidence from atomic force microscopy.  
AUTHOR(S): Lee, Ida (1); Evans, Barbara R.; Woodward, Jonathan  
CORPORATE SOURCE: (1) Electrical Engineering Department, University of Tennessee, Knoxville, TN, 37996-2100 USA  
SOURCE: Ultramicroscopy, (Feb., 2000) Vol. 82, No. 1-4, pp. 213-221.  
ISSN: 0304-3991.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB Two **cellulases** from *Trichoderma reesei* - an exoglucanase, CBH I, and an endoglucanase, EG II - alone and in combination were incubated with cotton fibers. The effects of the **cellulases** on the surfaces of the cotton fibers were examined by atomic force microscopy. At high magnification, the physical effects on the fibers caused by the two types of enzymes were considerably different. Treatment with CBH I resulted in the appearance of distinct pathways or tracks along the length of the microfibril. Treatment with EG II appeared to cause peeling and smoothing of the fiber surface. In combination, their effect was observed to be greatest when both enzymes were present simultaneously. When fibers smoothed by treatment with EG II were treated subsequently with CBH I, further evidence of path way formation caused by the action of CBH I along the fibers was observed. Incubation with a **cellulase** from *Thermotoga maritima* that lacks a cellulose binding domain had no effect on the surface of cotton fibers. These images provide the first physical evidence of differences in the effect of **cellulase** components action on the surface of cotton fibers and provide evidence for

the movement or tracking of CBH I along the fibers. The first AFM image of CBH I molecules are presented.

L4 ANSWER 10 OF 29 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE  
6

ACCESSION NUMBER: 2000:295957 BIOSIS  
DOCUMENT NUMBER: PREV200000295957  
TITLE: Carboxymethyl **cellulase** from *Thermotoga*  
*maritima*.  
AUTHOR(S): Mathur, Eric J.; Lam, David E.  
ASSIGNEE: Diversa Corporation  
PATENT INFORMATION: US 6008032 December 28, 1999  
SOURCE: Official Gazette of the United States Patent and Trademark  
Office Patents, (Dec. 28, 1999) Vol. 1229, No. 4, pp. No  
pagination. e-file.  
ISSN: 0098-1133.  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
AB A **purified** thermostable enzyme is derived from the eubacterium  
T. *maritima*. The enzyme has a molecular weight as determined by  
gel electrophoresis of about 35 kilodaltons and has **cellulase**  
activity. The enzyme can be produced from native or recombinant host cells  
and can be used to aid in the digestion of cellulose where desired.

L4 ANSWER 11 OF 29 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1999:635426 CAPLUS  
DOCUMENT NUMBER: 131:271022  
TITLE: Carboxymethylcellulase from *Thermotoga*  
*maritima*  
INVENTOR(S): Mathur, Eric J.; Lam, David E.  
PATENT ASSIGNEE(S): Diversa Corporation, USA  
SOURCE: U.S., 14 pp.  
CODEN: USXXAM  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 4  
PATENT INFORMATION:

**BEST AVAILABLE COPY**

| PATENT NO.    | KIND | DATE     | APPLICATION NO. | DATE     |
|---------------|------|----------|-----------------|----------|
| US 5962258    | A    | 19991005 | US 1995-518615  | 19950823 |
| US 6008032    | A    | 19991228 | US 1997-951889  | 19971016 |
| US 5925749    | A    | 19990720 | US 1998-66075   | 19980424 |
| US 6245547    | B1   | 20010612 | US 1999-472857  | 19991227 |
| US 2003044956 | A1   | 20030306 | US 2001-880729  | 20010612 |

PRIORITY APPLN. INFO.: US 1995-518615 A3 19950823  
US 1997-951889 A3 19971016  
US 1999-472857 A2 19991227

AB A **purified** thermostable enzyme is derived from the eubacterium  
T. *maritima*. The enzyme has a mol. wt. as detd. by gel  
electrophoresis of .apprx.35 kilodaltons and has **cellulase**  
activity. The enzyme can be produced from native or recombinant host  
cells and can be used to aid in the digestion of cellulose where desired.  
REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 12 OF 29 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1999:450916 CAPLUS  
DOCUMENT NUMBER: 131:84840  
TITLE: Carboxymethyl-**cellulase** and its gene from  
*Thermotoga* *maritima*  
INVENTOR(S): Mathur, Eric J.; Lam, David E.  
PATENT ASSIGNEE(S): Diversa Corporation, USA  
SOURCE: U.S., 13 pp.



CODEN: USXXAM  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 4  
PATENT INFORMATION:

| PATENT NO. | KIND | DATE     | APPLICATION NO. | DATE     |
|------------|------|----------|-----------------|----------|
| US 5925749 | A    | 19990720 | US 1998-66075   | 19980424 |
| US 5962258 | A    | 19991005 | US 1995-518615  | 19950823 |

PRIORITY APPLN. INFO.: US 1995-518615 A1 19950823

AB A **purified** thermostable enzyme is derived from the eubacterium *T. maritima*. The enzyme has a mol. wt. as detd. by gel electrophoresis of .apprx.35 kDa and has **cellulase** activity. The enzyme can be produced from native or recombinant host cells and can be used to aid in the digestion of cellulose where desired. The genomic gene sequence of the **cellulase** allows design of oligonucleotide probes.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 13 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI

ACCESSION NUMBER: 1999:201560 SCISEARCH

THE GENUINE ARTICLE: 173FW

TITLE: Comparing the thermodynamic stabilities of a related thermophilic and mesophilic enzyme

AUTHOR: Beadle B M; Baase W A; Wilson D B; Gilkes N R; Shoichet B K (Reprint)

CORPORATE SOURCE: NORTHWESTERN UNIV, DEPT MOL PHARMACOL & BIOL CHEM, 303 E CHICAGO AVE, CHICAGO, IL 60611 (Reprint); NORTHWESTERN UNIV, DEPT MOL PHARMACOL & BIOL CHEM, CHICAGO, IL 60611; UNIV OREGON, INST MOL BIOL, EUGENE, OR 97403; CORNELL UNIV, BIOCHEM MOL & CELL BIOL SECT, ITHACA, NY 14853; UNIV BRITISH COLUMBIA, DEPT MICROBIOL, VANCOUVER, BC, CANADA

COUNTRY OF AUTHOR: USA; CANADA

SOURCE: BIOCHEMISTRY, (23 FEB 1999) Vol. 38, No. 8, pp. 2570-2576. Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036.

ISSN: 0006-2960.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 32

**BEST AVAILABLE COPY**

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Several models have been proposed to explain the high temperatures required to denature enzymes from thermophilic organisms; some involve greater maximum thermodynamic stability for the thermophile, and others do not. To test these models, we reversibly melted two analogous protein domains in a two-state manner. E2(cd) is the **isolated** catalytic domain of **cellulase** E2 from the thermophile *Thermomonospora fusca*. CenA(P30) is the analogous domain of the **cellulase** CenA from the mesophile *Cellulomonas fimi*. When reversibly denatured in a common buffer, the thermophilic enzyme E2(cd) had a temperature of melting (T-m) of 72.2 degrees C, a van't Hoff enthalpy of unfolding (Delta H-VH) of 190 kcal/mol, and an entropy of unfolding (Delta S-u) of 0.55 kcal/(mol.K); the mesophilic enzyme CenA(P30) had a T-m of 56.3 degrees C, a Delta H-VH of 107 kcal/mol, and a Delta S-u of 0.32 kcal/(mol.K). The higher Delta H-VH and Delta S-u values for E2(cd) suggest that its free energy of unfolding (Delta G(u)) has a steeper dependence on temperature at the T-m than CenA(P30). This result supports models that predict a greater maximum thermodynamic stability for thermophilic enzymes than for their mesophilic counterparts. This was further explored by urea denaturation. Under reducing conditions at 30 degrees C, E2(cd) had a concentration of melting (C-m) of 5.2 M and a Delta G(u) of 11.2 kcal/mol;

CenA(P30) had a C-m of 2.6 M and a Delta G(u) of 4.3 kcal/mol, Under nonreducing conditions, the C-m and he, of CenA(P30) were increased to 4.5 M and 10.8 kcal/mol at 30 degrees C; the C-m for E2(cd) was increased to at least 7.4 M at 32 degrees C. We were unable to determine a Delta G(u) value for E2(cd) under nonreducing conditions due to problems with reversibility. These data suggest that E2(cd) attains its greater thermal stability (Delta T-m = 15.8 degrees C) through a greater thermodynamic stability (Delta Delta G(u) = 6.9 kcal/mol) compared to its mesophilic analogue CenA(P30).

L4 ANSWER 14 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI

ACCESSION NUMBER: 1999:531603 SCISEARCH

THE GENUINE ARTICLE: 212FU

TITLE: Extremophiles as a source of novel enzymes for industrial application

AUTHOR: Niehaus F; Bertoldo C; Kahler M; Antranikian G (Reprint)

CORPORATE SOURCE: TECH UNIV HAMBURG, INST TECH MICROBIOL, DENICKESTR 15, D-21071 HAMBURG, GERMANY (Reprint); TECH UNIV HAMBURG, INST TECH MICROBIOL, D-21071 HAMBURG, GERMANY

COUNTRY OF AUTHOR: GERMANY

SOURCE: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (JUN 1999) Vol. 51, No. 6, pp. 711-729.

Publisher: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010.

ISSN: 0175-7598.

DOCUMENT TYPE: General Review; Journal

FILE SEGMENT: LIFE; AGRI

LANGUAGE: English

REFERENCE COUNT: 200

**BEST AVAILABLE COPY**

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Extremophilic microorganisms are adapted to survive in ecological niches such as at high temperatures, extremes of pH, high salt concentrations and high pressure. These microorganisms produce unique biocatalysts that function under extreme conditions comparable to those prevailing in various industrial processes. Some of the enzymes from extremophiles have already been **purified** and their genes successfully **cloned** in mesophilic hosts. In this review we will briefly discuss the biotechnological significance of extreme thermophilic (optimal growth 70-80 degrees C) and hyperthermophilic (optimal growth 85-100 degrees C) archaea and bacteria. In particular, we will focus on selected extracellular-polymer-degrading enzymes, such as amylases, pullulanases, cyclodextrin glycosyltransferases, **cellulases**, xylanases, chitinases, proteinases and other enzymes such as esterases, glucose isomerases, alcohol dehydrogenases and DNA-modifying enzymes with potential use in food, chemical and pharmaceutical industries and in environmental biotechnology.

L4 ANSWER 15 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI

ACCESSION NUMBER: 1999:147095 SCISEARCH

THE GENUINE ARTICLE: 166HH

TITLE: Molecular diversity of thermophilic cellulolytic and hemicellulolytic bacteria

AUTHOR: Bergquist P L (Reprint); Gibbs M D; Morris D D; Teo V S; Saul D J; Moran H W

CORPORATE SOURCE: MACQUARIE UNIV, SCH BIOL SCI, N RYDE, NSW 2109, AUSTRALIA (Reprint); UNIV AUCKLAND, SCH MED, DEPT MOL MED, AUCKLAND, NEW ZEALAND; UNIV AUCKLAND, SCH BIOL SCI, CTR GENE TECHNOL, AUCKLAND, NEW ZEALAND; UNIV WAIKATO, THERMOPHILE RES UNIT, HAMILTON, NEW ZEALAND

COUNTRY OF AUTHOR: AUSTRALIA; NEW ZEALAND

SOURCE: FEMS MICROBIOLOGY ECOLOGY, (FEB 1999) Vol. 28, No. 2, pp. 99-110.

Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.

ISSN: 0168-6496.  
DOCUMENT TYPE: General Review; Journal  
FILE SEGMENT: LIFE; AGRI  
LANGUAGE: English  
REFERENCE COUNT: 48

**BEST AVAILABLE COPY**

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Many thermophilic bacteria belong to groups with deep phylogenetic lineages and ancestral forms were established before the occurrence of eucaryotes that produced cellulose and hemicellulose. Thus they may have acquired their beta-glycanase genes from more recent mesophilic bacteria. Most research has focussed on extremely thermophilic eubacteria growing above 65 degrees C under anaerobic conditions. Only recently have aerobic cellulolytic thermophiles been described from widely separated lineages (for example, *Rhodothermus marinus*, *Caldibacillus cellulovorans*). Many thermophilic bacteria produce **cellulases** and xylanases that have novel structures, with additional protein domains not identified with their catalytic activity. Many of these enzymes are multifunctional and code for more than one catalytic activity. This type of enzyme structure was first identified in the extreme thermophile *Caldicellulosiruptor* *caccharolyticus*. There is a general relatedness evident between catalytic domains, cellulose binding domains and other ancillary domains, which suggests that there may have been significant lateral gene transfer in the evolution of these microorganisms. Detailed molecular studies show that there is variation in the sequences of these related but not identical genes from taxonomically widely-separated organisms. (C) 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

L4 ANSWER 16 OF 29 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

ACCESSION NUMBER: 1998-08934 BIOTECHDS

TITLE: Glycosidase enzymes from organisms of the genera e.g. *Thermotoga*, *Thermococcus*, etc.;  
recombinant enzyme preparation and use in glucose preparation for the food, pharmaceutical, surfactant and textile industry

AUTHOR: Bylina E J; Swanson R V; Mathur E J; Lam D E

PATENT ASSIGNEE: Diversa

LOCATION: San Diego, CA, USA.

PATENT INFO: WO 9824799 11 Jun 1998

APPLICATION INFO: WO 1997-US22623 8 Dec 1997

PRIORITY INFO: US 1997-56916 10 Oct 1997; US 1996-56916 6 Dec 1996

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 1998-362407 [31]

AB A new nucleic acid encoding protein (I) (protein sequence and DNA sequence or RNA sequence specified) can be contained on a vector and used to transform a host cell for production of recombinant (I). (I) is used to produce glucose from soluble cell oligosaccharides in e.g. waste, food, feed or surfactants, for use in food, pharmaceutical, textile and surfactant industries. (I) is preferably a glycosidase from *Desulfurococcus* sp. M11TL, *Thermotoga* sp. OC1/4V-33BG, *Thermotoga maritima* MSB8 or MSB8-6GP2, *Staphylococcus marinus* F1-12G, *Thermococcus* sp. 9N2-31B/G, *Thermococcus alcaliphilus* AEDIII2RA, *Thermococcus chitinophagus* GC74-22G, *Pyrococcus furiosus* VC1-7G1, a **cellulase** (EC-3.2.1.4) from *Bankia gouldi* 37GP1 or *Thermotoga* sp. OC1/4V, an alpha-galactosidase (EC-3.2.1.22) from *T. maritima* 6GC2, an endo-1,4-beta-D-mannanase (EC-3.2.1.78) from *T. maritima* 6GP2, a pullulanase (EC-3.2.1.41) from *T. maritima* 6GP2, a beta-mannosidase (EC-3.2.1.25) from AEPII-1a or unidentified protein from *T. maritima* MSB8-6GB4, *Pyrococcus furiosus* VC1-7EG1 or *Bankia gouldi* 37GP4. (92pp)

L4 ANSWER 17 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI

ACCESSION NUMBER: 1998:942580 SCISEARCH

THE GENUINE ARTICLE: 145XB

TITLE: **Purification**, characterization, and molecular analysis of thermostable **cellulases** Cella and CelB from *Thermotoga neapolitana*

AUTHOR: Bok J D; Yernool D A; Eveleigh D E (Reprint)

CORPORATE SOURCE: RUTGERS STATE UNIV, COOK COLL, DEPT MICROBIOL & BIOCHEM, 76 LIPMAN DR, NEW BRUNSWICK, NJ 08901 (Reprint); RUTGERS STATE UNIV, COOK COLL, DEPT MICROBIOL & BIOCHEM, NEW BRUNSWICK, NJ 08901

COUNTRY OF AUTHOR: USA

SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (DEC 1998) Vol. 64, No. 12, pp. 4774-4781.  
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.  
ISSN: 0099-2240.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE; AGRI

LANGUAGE: English

REFERENCE COUNT: 54

## BEST AVAILABLE COPY

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Two thermostable endocellulases, Cella and CelB, were **purified** from *Thermotoga neapolitana*. Cella (molecular mass, 29 kDa; pI 4.6) is optimally active at pH 6.0 at 95 degrees C, while CelB (molecular mass, 30 kDa; pI 4.1) has a broader optimal pH range (pH 6.0 to 6.6) at 106 degrees C. Both enzymes are characterized by a high level of activity (high V-max value and lent apparent K-m value) with carboxymethyl cellulose; the specific activities of Cella and CelB are 1,219 and 1,536 U/mg, respectively. With p-nitrophenyl cellobioside the V-max values of Cella and CelB are 69.2 and 18.4 U/mg, respectively, while the K-m values are 0.97 and 0.3 mM, respectively. The major end products of cellulose hydrolysis, glucose and cellobiose, competitively inhibit Cella, and CelB. The K-i values for Cella are 0.44 M for glucose and 2.5 mM for cellobiose; the K-i values for CelB are 0.2 M for glucose and 1.16 mM for cellobiose. CelB preferentially cleaves larger cellooligomers, producing cellobiose as the end product; it also exhibits significant transglycosylation activity. This enzyme is highly thermostable and has half-lives of 130 min at 106 degrees C and 26 min at 110 degrees C. A single **clone** encoding the celA and celB genes was identified by screening a *T. neapolitana* genomic library in *Escherichia coli*. The celA gene encodes a 257-amino-acid protein, while celB encodes a 274-amino-acid protein. Both proteins belong to family 12 of the glycosyl hydrolases, and the two proteins are 60% similar to each other. Northern blots of *T. neapolitana* mRNA revealed that celA and celB are monocistronic messages, and both genes are inducible by cellobiose and are repressed by glucose.

L4 ANSWER 18 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI

ACCESSION NUMBER: 1998:313351 SCISEARCH

THE GENUINE ARTICLE: ZH504

TITLE: **Cloning**, sequencing and overexpression of a *Rhodothermus marinus* gene encoding a thermostable **cellulase** of glycosyl hydrolase family 12

AUTHOR: Halldorsdottir S; Thorolfssdottir E T; Spilliaert R; Johansson M; Thorbjarnardottir S H; Palsdottir A; Hreggvidsson G O; Kristjansson J K; Holst O; Eggertsson G (Reprint)

CORPORATE SOURCE: UNIV ICELAND, INST BIOL, MOL GENET LAB, IS-108 REYKJAVIK, ICELAND (Reprint); UNIV ICELAND, INST BIOL, MOL GENET LAB, IS-108 REYKJAVIK, ICELAND; TECHNOL INST ICELAND, DEPT BIOTECHNOL, IS-112 REYKJAVIK, ICELAND; LUND UNIV, CTR CHEM & CHEM ENGN, S-22100 LUND, SWEDEN

COUNTRY OF AUTHOR: ICELAND; SWEDEN

SOURCE: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (MAR 1998) Vol. 49, No. 3, pp. 277-284.  
Publisher: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY

10010.  
 ISSN: 0175-7598.  
 DOCUMENT TYPE: Article; Journal  
 FILE SEGMENT: LIFE; AGRI  
 LANGUAGE: English  
 REFERENCE COUNT: 36

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB A gene library from the thermophilic eubacterium *Rhodothermus marinus*, strain ITI 378, was constructed in pUC18 and transformed into *Escherichia coli*. Of 5400 transformants, 3 were active on carboxymethylcellulose. Three plasmids conferring **cellulase** activity were **purified** and were all found to contain the same **cellulase** gene, *celA*. The open reading frame for the *celA* gene is 780 base pairs and encodes a protein of 260 amino acids with a calculated molecular mass of 28.5 kDa. The amino acid sequence shows homology with **cellulases** in glycosyl hydrolase family 12. The *celA* gene was overexpressed in *E. coli* when the pET23, T7 phage RNA polymerase system was used. The enzyme showed activity on carboxymethylcellulose and lichenan, but not on birch xylan or laminarin. The expressed enzyme had six terminal histidine residues and was **purified** by using a nickel nitrilotriacetate column. The enzyme had a pH optimum of 6-7 and its highest measured initial activity at 100 degrees C. The heat stability of the enzyme was increased by removal of the histidine residues. It then retained 75% of its activity after 8 h at 90 degrees C.

L4 ANSWER 19 OF 29 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 7  
 ACCESSION NUMBER: 1997:776184 CAPLUS  
 DOCUMENT NUMBER: 128:72370  
 TITLE: Endoglucanases gene sequences from thermophilic archaeal bacteria  
 INVENTOR(S): Lam, David E.; Mathur, Eric J.  
 PATENT ASSIGNEE(S): Recombinant Biocatalysis, Inc., USA; Lam, David E.; Mathur, Eric J.  
 SOURCE: PCT Int. Appl., 125 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 2  
 PATENT INFORMATION:

**BEST AVAILABLE COPY**

| PATENT NO.  | KIND | DATE     | APPLICATION NO. | DATE     |
|---|------|----------|-----------------|----------|
| WO 9744361  | A1   | 19971127 | WO 1997-US8793  | 19970522 |
| W: AU, CA, IL, JP, US   |      |          |                 |          |
| RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE    |      |          |                 |          |
| US 5789228  | A    | 19980804 | US 1996-651572  | 19960522 |
| AU 9732852  | A1   | 19971209 | AU 1997-32852   | 19970522 |
| AU 719444   | B2   | 20000511 |                 |          |
| EP 923608   | A1   | 19990623 | EP 1997-928650  | 19970522 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI |      |          |                 |          |
| JP 2000512842   | T2   | 20001003 | JP 1997-542781  | 19970522 |
| US 6074867  | A    | 20000613 | US 1997-951086  | 19971015 |
| PRIORITY APPLN. INFO.: US 1996-651572 A2 19960522                         |      |          |                 |          |
| WO 1997-US8793 W 19970522   |      |          |                 |          |

AB The invention provides a **purified** thermostable enzyme derived from the archaeal bacterium AEP111a. The enzyme has a mol. wt. of .apprx.60.9 kDa and has **cellulase** activity. The enzyme can be produced from native or recombinant host cells and can be used to aid in the digestion of cellulose where desired. Also included are an addnl. 23 genes and their encoded endoglucanases having homol. to the AEP111a enzyme. The **cellulases** enzymes may be used for degrdn. of cellulose for the conversion of plant biomass into fuels and chems., for use in detergents, the textile industry, in animal feed, in waste

treatment, and in the fruit juice/brewing industry for the clarification and extn. of juices.

L4 ANSWER 20 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI  
ACCESSION NUMBER: 97:854403 SCISEARCH  
THE GENUINE ARTICLE: YF644  
TITLE: Characterization of the xylanolytic enzyme system of the extreme thermophilic anaerobic bacteria *Thermotoga maritima*, *T. neapolitana*, and *T. thermarum*  
AUTHOR: Sunna A; Puls J; Antranikian G (Reprint)  
CORPORATE SOURCE: TECH UNIV HAMBURG, ARBEITSBEREICH BIOTECHNOL 1, DENICKESTR 15, D-21073 HAMBURG, GERMANY (Reprint); TECH UNIV HAMBURG, ARBEITSBEREICH BIOTECHNOL 1, D-21073 HAMBURG, GERMANY; BUNDESFORSCH ANSTALT FORST & HOLZWIRTSCH, INST HOLZCHEM, D-21031 HAMBURG, GERMANY  
COUNTRY OF AUTHOR: GERMANY  
SOURCE: COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY A-PHYSIOLOGY, (NOV 1997) Vol. 118, No. 3, pp. 453-461.  
Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD, ENGLAND OX5 1GB.  
ISSN: 0300-9629.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE; AGRI  
LANGUAGE: English  
REFERENCE COUNT: 44

**BEST AVAILABLE COPY**

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The extreme thermophilic anaerobic bacteria *Thermotoga maritima*, *T. neapolitana*, and *T. thermarum* when grown on xylan produce extremely thermoactive xylanases, beta-xylosidases and alpha-arabinofuranosidases. Most of these enzymes are active over a broad temperature and pH range, namely between 40 and 110 degrees C and pH 4 to 9. The xylanases are active towards soluble, as well as insoluble xylan. Unlike the enzyme system of *T. maritima* and *T. thermarum*, the xylanase activity of *T. neapolitana* is activated (up to 211%) in the presence of Ca<sup>2+</sup>, Mg<sup>2+</sup>, Co<sup>2+</sup>, and Mn<sup>2+</sup>. The xylanolytic enzyme system of *T. thermarum* is inactive towards cellulose. Xylan hydrolysis experiments indicate the presence of endoxylanases in the enzyme preparation of all strains investigated, with the main products being xylobiose, xylotriose, and larger xylooligosaccharides. (C) 1997 Elsevier Science Inc.

L4 ANSWER 21 OF 29 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 8  
ACCESSION NUMBER: 1996:599328 CAPLUS  
DOCUMENT NUMBER: 125:268866  
TITLE: Analysis of a *Thermotoga maritima* DNA fragment encoding two similar thermostable cellulases, CelA and CelB, and characterization of the recombinant enzymes  
AUTHOR(S): Liebl, Wolfgang; Ruile, peter; Bronnenmeier, Karin; Riedel, Katrin; Lottspeich, Freidrich; Greif, Ingrid  
CORPORATE SOURCE: Inst. Mikrobiol., Tech. Univ. Muenchen, Munich, D-80290, Germany  
SOURCE: Microbiology (Reading, United Kingdom) (1996), 142(9), 2533-2542  
CODEN: MROBEO; ISSN: 1350-0872  
PUBLISHER: Society for General Microbiology  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Recombinant *Escherichia coli* clones displaying thermostable .beta.-glucanase activity were isolated from two different gene libraries of the hyperthermophilic bacterium *Thermotoga maritima* MSB8 (DSM 3109), and the nucleotide sequence of a 1,4-.beta.-glucanase gene designated celA was detd. Amino-terminal sequencing of cellulase I previously detected in *T. maritima* cells indicated that the CelA gene encodes this .beta.-glucanase, which is now

designated CelA. CelA, which has a calcd. mol. mass of 29,732 Da, was **purified** from a recombinant *e. coli* strain to apparent homogeneity as judged by SDS-PAGE with a 44% yield. The enzyme was most active against sol. substrates such as mixed-linkage .beta.-glucan and CM-cellulose. CelA displayed remarkable thermostability, which was enhanced in the presence of high concns. of salt. Downstream of the *celA* gene the authors found a second open reading frame, *celB*, whose nucleotide sequence was 58% identical to *celA*. Exptl. proof that *celB* also encodes a .beta.-glucanase was obtained by sepn. from CelA and expression in *E. coli* under the control of an efficient host promoter. According to the deduced amino acid sequences, CelB, in contrast to CelA, contains a signal peptide at the amino terminus. CelB and CelA had similar substrate specificities and temp. optima, but differed in their pH optima. Also, the addn. of salt had a less stabilizing effect on CelB than on CelA. Nine 30 bp direct repeats, each itself representing a sequence with imperfect dyad symmetry, were detected upstream of the *CelA-celB cellulase* gene cluster.

L4 ANSWER 22 OF 29 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 9  
 ACCESSION NUMBER: 1995:479619 CAPLUS  
 DOCUMENT NUMBER: 122:259425  
 TITLE: **Purification of Thermotoga maritima**  
 enzymes for the degradation of cellulosic materials  
 AUTHOR(S): Bronnenmeier, Karin; Kern, Anja; Liebl, Wolfgang;  
 Staudenbauer, Walter L.  
 CORPORATE SOURCE: Institut fur Mikrobiologie, Technische Universitat  
 Munchen, Munich, 80 290, Germany  
 SOURCE: Applied and Environmental Microbiology (1995), 61(4),  
 1399-407  
 CODEN: AEMIDF; ISSN: 0099-2240  
 PUBLISHER: American Society for Microbiology  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English

AB A sepn. procedure for the anal. of the enzyme components of the hyperthermophilic bacterium *Thermotoga maritima* involved in cellulose and xylan degrdn. was developed. Resoln. of the enzymes was achieved by a combination of fast-protein liq. chromatog. anion exchange and hydrophobic interactions chromatog. Enzyme fractions were assayed for hydrolysis of Avicel, CM-cellulose (CMC), .beta.-glucan, laminarin, xylan, p-nitrophenyl-.beta.-D-glucoside, p-nitrophenyl-.beta.-D-cellobioside, p-nitrophenyl-.beta.-D-xyloside, p-nitrophenyl-.alpha.-L-arabinofuranoside, and 4-O-methyl-glucuronosyl-xylotriase. The activities of two **cellulases**, one laminarinase, one xylanase, two putative .beta.-D-xylosidases, .alpha.-D-glucuronidase, and .alpha.-L-arabinosidase were identified. Because of their selective retardation of a Superdex gel filtration column, the two **cellulases** could be **purified** to homogeneity. According to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, mol. masses of 27 and 29 kDa, resp., were detd. for **cellulase I** and **cellulase II**. Maximal activities of both enzymes were obsd. at 95 .degree.C between pH 6.0 and 7.5. In the presence of 2.5 M NaCl the **purified** enzymes retained about 90% of their initial activities after a 6-h incubation at 80 .degree.C. On the basis of its activity towards CMC, **cellulase I** was classified as endo-.beta.-1,4-glucanase. **Cellulase II** was able to attack Avicel in addn. to CMC, .beta.-glucan, and p-nitrophenyl-.beta.-D-cellobioside. It releases cellobiose and cellotriose from Avicel. The latter product is further cleaved into glucose and cellobiose. **Cellulase II** may therefore be classified as exo-.beta.-1,4-glucanase.

L4 ANSWER 23 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI  
 ACCESSION NUMBER: 94:155837 SCISEARCH  
 THE GENUINE ARTICLE: MY351  
 TITLE: DOMAIN-STRUCTURE OF THE ACETOGENIUM-KIVUI SURFACE-LAYER

BEST AVAILABLE COPY

AUTHOR: REVEALED BY ELECTRON CRYSTALLOGRAPHY AND SEQUENCE-ANALYSIS  
 LUPAS A; ENGELHARDT H; PETERS J; SANTARIUS U; VOLKER S;  
 BAUMEISTER W (Reprint)  
 CORPORATE SOURCE: MAX PLANCK INST BIOCHEM, D-82152 MARTINSRIED, GERMANY  
 (Reprint); MAX PLANCK INST BIOCHEM, D-82152 MARTINSRIED,  
 GERMANY  
 COUNTRY OF AUTHOR: GERMANY  
 SOURCE: JOURNAL OF BACTERIOLOGY, (MAR 1994) Vol. 176, No. 5, pp.  
 1224-1233.  
 ISSN: 0021-9193.  
 DOCUMENT TYPE: Article; Journal  
 FILE SEGMENT: LIFE  
 LANGUAGE: ENGLISH  
 REFERENCE COUNT: 32

## BEST AVAILABLE COPY

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The three-dimensional structure of the *Acetogenium kivui* surface layer  
 (S-layer) has been determined to a resolution of 1.7 nm by electron  
 crystallographic techniques. Two independent reconstructions were made  
 from layers negatively stained with uranyl acetate and  
 Na-phosphotungstate. The S-layer has p6 symmetry with a center-to-center  
 spacing of approximately 19 nm. Within the layer, six monomers combine to  
 form a ring-shaped core surrounded by a fenestrated rim and six spokes  
 that point towards the axis of threefold symmetry and provide lateral  
 connectivity to other hexamers in the layer. The structure of the *A. kivui*  
 S-layer protein is very similar to that of the *Bacillus brevis* middle wall  
 protein, with which it shares an N-terminal domain of homology. This  
 domain is found in several other extracellular proteins, including the  
 S-layer proteins from *Bacillus sphaericus* and *Thermus thermophilus*, *Omp*  
*alpha* from *Thermotoga maritima*, an alkaline cellulase  
 from *Bacillus* strain KSM-635, and xylanases from *Clostridium thermocellum*  
 and *Thermoanaerobacter saccharolyticum*, and may serve to anchor these  
 proteins to the peptidoglycan. To our knowledge, this is the first example  
 of a domain conserved in several S-layer proteins.

L4 ANSWER 24 OF 29 SCISEARCH COPYRIGHT 2003 THOMSON ISI

ACCESSION NUMBER: 94:150772 SCISEARCH

THE GENUINE ARTICLE: MY168

TITLE: COMPARATIVE AMINO-ACID-SEQUENCE ANALYSIS OF THERMOTOGA-  
**MARITIMA** BETA-GLUCOSIDASE (BGLA) DEDUCED FROM THE  
 NUCLEOTIDE-SEQUENCE OF THE GENE INDICATES DISTANT  
 RELATIONSHIP BETWEEN BETA-GLUCOSIDASES OF THE BGA FAMILY  
 AND OTHER FAMILIES OF BETA-1,4-GLYCOSYL HYDROLASES

AUTHOR: LIEBL W (Reprint); GABELSBERGER J; SCHLEIFER K H  
 CORPORATE SOURCE: TECH UNIV MUNICH, LEHRSTUHL MIKROBIOL, ARCSSTR 21,  
 D-80290 MUNICH, GERMANY (Reprint)  
 COUNTRY OF AUTHOR: GERMANY  
 SOURCE: MOLECULAR & GENERAL GENETICS, (JAN 1994) Vol. 242, No. 1,  
 pp. 111-115.  
 ISSN: 0026-8925.  
 DOCUMENT TYPE: Note; Journal  
 FILE SEGMENT: LIFE  
 LANGUAGE: ENGLISH  
 REFERENCE COUNT: 21

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The primary structure of the *bglA* gene region encoding a  
 beta-glucosidase of *Thermotoga maritima* strain MSB8 was  
 determined. The *bglA* gene has the potential to code for a polypeptide of  
 446 amino acids with a predicted molecular mass of 51545 Da. The *T.*  
*maritima* beta-glucosidase (*BglA*) was overexpressed in *E. coli* at a  
 level comprising approximately 15-20% of soluble cellular protein. Based  
 on its amino acid sequence, as deduced from the nucleotide sequence of the  
 gene, *BglA* can be classified as a broad-specificity beta-glucosidase and  
 as a member of the beta-glucosidase family BGA, in agreement with the  
 results of enzymatic characterization of the recombinant protein.



Comparative sequence analysis revealed distant amino acid sequence similarities between BGA family beta-glucosidases, a beta-xylosidase, beta-1,4-glycanases of the enzyme family F (mostly xylanases), and other families of beta-1,4-glycosyl hydrolases. This result indicates that BGA beta-glucosidases may comprise one enzyme family within a large 'enzyme order' of retaining beta-glycosyl hydrolases, and that the members of these enzyme groups may be inter-related at the level of active site architecture and perhaps even on the level of overall three-dimensional fold.

L4 ANSWER 25 OF 29 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 10  
 ACCESSION NUMBER: 1995:225667 CAPLUS  
 DOCUMENT NUMBER: 122:154681  
 TITLE: **Cellulase** and xylanase systems of *Thermotoga neapolitana*  
 AUTHOR(S): Bok, Jin Duck; Goers, Steven K.; Eveleigh, Douglas E.  
 CORPORATE SOURCE: Biochem. and Microbiol., Rutgers Univ., New Brunswick, NJ, 08903-0231, USA  
 SOURCE: ACS Symposium Series (1994), 566 (Enzymatic Conversion of Biomass for Fuels Production), 54-65  
 CODEN: ACSMC8; ISSN: 0097-6156  
 PUBLISHER: American Chemical Society  
 DOCUMENT TYPE: Journal; General Review  
 LANGUAGE: English

AB A review with 67 refs. *Thermotoga neapolitana* and *T. maritima*, hyperthermophiles that grow optimally at 80.degree.C, have very similar **cellulase** systems based on SDS-PAGE/zymogram anal. *T. neapolitana* has at least three types of **cellulase** components, including an aryl .beta.-glucosidase (BglA), a .beta.-glucosidase with cellobiohydrolase activity (BglB) and endoglucanase (EndoA and B). The Sephadex adsorption properties of EndoB allowed a facile **purifn.** from both *T. neapolitana* and *T. maritima*. All enzymes exhibited very high thermostability, e.g. EndoB  $t_{1/2}$ =8 h at 100.degree.C. The specific activities (CMC U/mg) of both endoglucanases are very high: 1,200 for EndoA and 1,500 for EndoB. These properties make *Thermotoga* endoglucanases potentially useful for industrial application. To facilitate prodn., these endoglucanase genes and those of BglA and B have been **cloned** in *E. coli*. Furthermore, an endo-acting .beta.-1,4-xylanase from *T. neapolitana* was discovered and found to have remarkable thermal stability ( $t_{1/2}$ =130 h at 82.degree.C), and pH and temp. optima resp. of 5.5 and 85.degree.C. This xylanase has little endoglucanase activity and has potential application for biol. bleaching in the pulp and paper industry.

L4 ANSWER 26 OF 29 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
 ACCESSION NUMBER: 1992-09382 BIOTECHDS  
 TITLE: **Cloning** and characterization of an endoglucanase and a xylanase from the hyperthermophilic eubacterium *Thermotoga maritima*; **cellulase** complex characterization and thermostable CM-**cellulase** gene **cloning** and expression (conference abstract)  
 AUTHOR: Bok J D; Goers S K; Eveleigh D E  
 LOCATION: Rutgers University, New Brunswick, NJ 08903, USA.  
 SOURCE: Abstr.Gen.Meet.Am.Soc.Microbiol.; (1992) 92 Meet., 312  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English

AB Cellulose and hemicellulose biomass is a central energy reserve for many ecological systems. *Thermotoga maritima*, an anaerobic, hyperthermophilic (growth at 90 deg) marine eubacterium is cellulolytic. The hydrolases of this bacterium have been characterized, and include 1 endoglucanase (CM-**cellulase**, EC-3.2.1.4), up to 3 endo-1,4-beta-D-xylanases (EC-3.2.1.8), 1 beta-glucosidase (EC-3.2.1.21)/cellobiohydrolase (EC-3.2.1.91) and 1 beta-glucosidase.

The CM-cellulase was purified to homogeneity and characterized. It had a temp. optimum of 90-95 deg at pH 5, with specific activity of 130 U/mg. It was a small (mol.wt. 26,000) acidic enzyme with an isoelectric point at 3.6. A gene bank from *T. maritima* was constructed in *Escherichia coli* using plasmid pUC119. Gene egI encoding cellulase was cloned and expressed. The gene product was purified and characterized. Cloned and native enzymes had almost identical characteristics. 1 Xylanase has also been cloned and characterized. (0 ref)

L4 ANSWER 27 OF 29 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

ACCESSION NUMBER: 1991-07888 BIOTECHDS

TITLE: Cloning genes from thermophiles: a review of current research; (conference paper)

AUTHOR: McHale R H; Saul D; Ashby M; Luthi E; Whitefield J; Bergquist P L

LOCATION: Thermophile Genetics Research Group, Centre for Gene Technology, Department of Cellular and Molecular Biology, University of Auckland, New Zealand.

SOURCE: Ferment.Technol.Ind.Appl.; (1990) 9-14

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The cloning of genes from thermophilic microorganisms was reviewed. A number of genes encoding thermostable enzymes involved in degradation of cellulose and xylan have been cloned, sequenced and expressed in *Escherichia coli*, and in some cases, in *Bacillus subtilis*. These include the beta-glucosidase (EC-3.2.1.21), cellulase (EC-3.2.1.4), cellobiohydrolase (EC-3.2.1.91), endo-1,4-beta-D-xylanase (EC-3.2.1.8) and beta-xylosidase (EC-3.2.1.37) genes of *Caldocellum saccharolyticum*. The pullulanase (EC-3.2.1.41) gene of this thermophilic bacterium has also been cloned, sequenced and expressed in *E. coli*. The ease of expression of the genes in *E. coli* has not been found for genes from other thermophiles, such as *Thermotoga maritima*. Alternative strategies have therefore been adopted and include the use of the polymerase chain reaction to amplify specific gene sequences, immunological assays and the development of a gene transfer system in *Thermus* sp. Genes for 16S ribosomal RNAs from thermophiles have been cloned and sequenced as an aid to taxonomic and phylogenetic investigations. (23 ref)

L4 ANSWER 28 OF 29 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2003)

ACCESSION NUMBER: 83:62865 AGRICOLA

DOCUMENT NUMBER: IND83051918

TITLE: Purification of three cellulases from the xylophagous larvae of *Ergates faber* (Coleoptera:Cerambycidae) *Pinus maritima*.

AUTHOR(S): Chararas, C.; Eberhard, R.; Courtois, J.E.; Petek, F.

AVAILABILITY: DNAL (QL495.A1I57)

SOURCE: Insect biochemistry., 1983 Vol. 13, No. 2. p. 213-218

Publisher: Oxford : Pergamon Press.

ISSN: 0020-1790

NOTE: Includes references.

DOCUMENT TYPE: Article

FILE SEGMENT: Non-U.S. Imprint other than FAO

LANGUAGE: English

L4 ANSWER 29 OF 29 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1975:404972 CAPLUS

DOCUMENT NUMBER: 83:4972

TITLE: Production and activity of cellulolytic enzymes by

AUTHOR(S): higher fungi from marine and brackish waters  
Schaumann, K.  
CORPORATE SOURCE: Inst. Meeresforsch., Bremerhaven, Fed. Rep. Ger.  
SOURCE: Marine Biology (Berlin, Germany) (1974), 28(3), 221-35  
CODEN: MBIOAJ; ISSN: 0025-3162  
DOCUMENT TYPE: Journal  
LANGUAGE: German

AB Twenty isolations of higher marine fungi were examd. in regard to their cellulolytic capabilities. Application of the viscosimetric method produced detailed information on the cellulolytic activities of these fungi. However, only the Cx-component of the total cellulose complex could be estd. by this method. The most active species were *Dendryphiella salina*, *Chaetomium ramipilosum*, *Asteromyces cruciatus*, and *Humicola alopallonella*. Only very slight or no Cx-activity was obsd. in *Cirrenalia macrocephala*, *Monodictys pelagica*, and *Zalerion maritimum*. These findings reveal no correlation between the cellulolytic activity of the fungi in vitro and their frequency on wood substrates in situ. For example, differences in the intensity of Cx-cellulase prodn. of *D. salina* were caused by variations in salinity and compn. of the nutrient broth, especially by the kind of cellulose added for enzyme induction, and by the addn. or absence of glucose. Most of the Cx-cellulase produced was present in the cell-free culture-filtrate. Only a small quantity was absorbed by the cellulose particles or the fungal mycelia. Parallel to the mycelial growth, and accompanied by a shift in pH, the Cx-cellulase-activity rose continuously, attaining a max. after several weeks. During further cultivation, the max. remained more or less const. for a long period. Tests using different methods, e.g. cellulose powder-agar plates, proved unsuitable because of spreading hyphal growth, dark pigmentation, heavy sexual or asexual sporulation, and relatively low cellulase prodn. during short-culture periods.

BEST AVAILABLE COPY